[0011] In certain embodiments, the ratio of the first cell to the second cell is between about 1:10 and about 50:1. In certain embodiments, the ratio of the first cell to the second cell is between about 1:10 and about 10:1. In certain embodiments, the average expression of the second antigen on the second cell is at least about 1,000 molecules per cell. In certain embodiments, the average expression of the second antigen on the second cell is at least about 100,000 molecules per cell. In certain embodiments, the average distance between the first cell and the second cell is no more than about 0.3 mm. In certain embodiments, the average distance between the first cell and the second cell is no more than about 0.1 mm.

[0012] The presently disclosed subject matter is also directed to kits for determining cellular synapse formation, e.g., cellular synapse formation induced by a multispecific antibody that binds to a first antigen and a second antigen, where the first antigen is expressed by a first cell and the second antigen is expressed by a second cell. In certain embodiments, the kits of the present disclosure comprise (a) a first cell expressing the first antigen; (b) a second cell expressing the second antigen; and (c) means for measuring activation of the first cell. In certain embodiments, a cellular synapse is formed between the first cell and the second cell upon binding of the multispecific antibody to the first antigen and the second antigen. In certain embodiments, the cellular synapse formation activates the first cell.

[0013] The presently disclosed subject matter further provides systems for determining cellular synapse formation where the system comprises (a) a first cell expressing the first antigen; (b) a second cell expressing the second antigen; and (c) means for measuring activation of the first cell.

BRIEF DESCRIPTION OF THE FIGURES

[0014] FIG. **1** depicts the structure of the cellular synapse model, describing the binding of T cell dependent bispecific antibody (TDB) to B lymphoma cell and T-cell, and the formation of cellular synapse.

[0015] FIGS. **2**A-**2**B depict use of CD 69 and CD62L as biomarkers for T cell activation. FIG. **2**A depicts Jurkat T cells incubated with BJAB B cells and CD20/CD3 TDB stained for CD69 and CD62L expression. FIG. **2**B depicts that the percentage of T cells with increased CD69 or decreased CD62L was used to calculate % T cell activation opposite TDB concentration. Error bars indicate SEM.

[0016] FIGS. **3**A-**3**B depict detection of T cell activation. FIG. **3**A depicts that Jurkat T cells were incubated with BJAB B cells and CD20/CD3 TDB over a 24 hour time course. The percentage of activation as marked by CD69 increase or C62L decrease was calculated and plotted. FIG. **3**B depicts that Jurkat T cells were incubated with BJAB B cells and CD20/CD3 TDB concentration titration over a 4 hour time course. The decrease in CD62L expression was used to calculate percentage T cell activation. T cell activation was plotted opposite TDB concentration. Error bars indicate SEM.

[0017] FIG. **4** depicts detection of CD4 and CD8 T cell activation. Human PMBCs were incubated with CD20/CD3 TDB for 4 hours. The percentage of CD4 and CD8 T cell activation measured by C62L decrease was calculated and plotted. Error bars indicate SEM.

[0018] FIG. **5** depicts predicted versus observed cellular synapse. Black circles represent observed cellular synapse percentage (number of T cell with CD62L T cell activation marker normalized to total number of T cell) at various effector:target (E:T) cell ratio and CD20/CD3 TDB concentration. Gray circles represent model-predicted cellular synapse percentage.

[0019] FIG. **6** depicts that the T cells are more likely to be activated when B cells had a higher expression level of the antigen CD20. B cell R is CD20, the expression levels are 1,200 per cell, 1,400 per cell or 122,000 per cell in the test samples.

[0020] FIG. **7** depicts a simulation of 500 T cells and 500 B cell in 1 μL.

[0021] FIG. **8** depicts simulations of intracellular distance between T cells and B cells.

[0022] FIG. **9** depicts T cell activation at conditions of different B cell antigen expression (1,200 per cell, 1,400 per cell or 122,000 per cell) and intracellular distance between T cells and B cells (distance (Dx) from 0.04 mm to 0.30 mm).

DETAILED DESCRIPTION

[0023] 1. Definitions

[0024] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies and TDB antibodies), as well as antibody fragments so long as they exhibit the desired antigen-binding activity.

[0025] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments. In certain embodiments, the antibody fragment is a Fab molecule. In certain embodiments, the antibody fragment is a $F(ab')_2$ molecule.

[0026] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0027] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (C_H1, C_H2, and C_H3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody can be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0028] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains